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**L-lysine dose dependently delays gastric emptying and
increases intestinal fluid volume in humans and rats**

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“L-lysine dose dependently delays gastric emptying and increases intestinal fluid volume in humans and rats.”

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Lebenslauf

“L-lysine dose dependently delays gastric emptying and increases intestinal fluid volume in humans and rats”

Novel sensory inputs for the control of food intake and gastrointestinal function (GI) are of increasing interest due to the rapid increase in nutrition-related diseases. The essential amino acid L-lysine (Lys) was demonstrated to have a selective impact on food intake, gastric emptying, and intestinal transit in rats, thus indicating a potential novel direct sensory input to assess dietary protein content and quality. The aim of this study was to investigate the dose-dependent effect of Lys on human and rat GI function. Lys doses from 0–800 mg in rats and 0.5–7.5 g in humans were analysed for their effect on gastric emptying and GI secretion. Human GI function was assessed noninvasively using MRI, rat data were acquired using standard lethal measurement methods. Lys dose dependently delayed gastric emptying and stimulated GI secretion in rats as reflected by residual phenol red content and increased gastric wet weight. The dose-dependent delay in gastric emptying observed in rats was confirmed in humans with an increase in halftime of gastric emptying. Moreover, a dose-dependent increase in intestinal fluid accumulation was observed. No effect on alkaline tide, glucose concentration, haematocrit, or visceral sensations was detected. This translational study demonstrates comparable dose-dependent effects of intragastric Lys on GI function in humans and rats and suggests a broader role for individual amino acids in the control of GI motility and secretion in vivo.

Gastric emptying, gastrointestinal function, L-lysine, MRI

Zusammenfassung

Die Ernährungswissenschaft interessiert sich wie der Energiestoffwechsel, insbesondere die gastrointestinale Funktion (giF) und die Nahrungsaufnahme beeinflusst werden kann. Bei Rattenversuchen, zeigte die essentielle Aminosäure L-lysine (Lys) einen selektiven Einfluss auf die Nahrungsaufnahme und die Magenentleerung (ME) und den intestinalen Transit. Das Ziel dieser Studie war den dosisabhängigen Effekt von Lys auf den Verdauungstrakt im Menschen zu untersuchen. Es wurde die Wirkung von 0.5 bis 7.5 g Lys auf die ME und Magensekretion (MS) in gesunden Probanden ermittelt, ergänzend wurde auch der dosisabhängige Effekt von 0 bis 800 mg Lys in Ratten getestet. Die menschliche giF wurde nichtinvasiv mittels der Magnetresonanztomographie (MRT) quantifiziert. Lys verzögerte die ME dosisabhängig in den Probanden, hatte jedoch keinen Einfluss auf die MS. Zudem wurde mittels MRT eine dosisabhängige Zunahme der Dünndarmflüssigkeit festgestellt. Es konnte kein Effekt auf den Säure-Basenhaushalt, Glukosekonzentration, Hämatokrit oder viszerale Wahrnehmungen nachgewiesen werden. Die dosisabhängige Verzögerung der ME konnte in den Ratten bestätigt werden, wo zudem auch ein Effekt auf die MS detektiert wurde. Diese translatoren Studie weist auf eine vergleichbare dosisabhängige Wirkung von Lys auf die giF beim Menschen und der Ratte hin. Diese ersten Erkenntnisse lassen eine spezifische Rolle einzelner Aminosäuren bei der Kontrolle der Verdauung und Nahrungsaufnahme vermuten.

Magenentleerung, Gastrointestinale Funktion, L-lysine, MRI

L-lysine dose dependently delays gastric emptying and increases intestinal fluid volume in humans and rats

Running Title

The impact of L-lysine on GI function

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Abstract

Background: Novel sensory inputs for the control of food intake and gastrointestinal (GI) function are of increasing interest due to the rapid increase in nutrition related diseases. The essential amino acid L-lysine was demonstrated to have a selective impact on food intake, gastric emptying and intestinal transit in rats thus indicating a potential novel direct sensory input to assess dietary protein content and quality. The aim of this study was to assess translational aspects of this finding and to investigate the dose-dependent effect of L-lysine on human and rat GI function. **Methods:** L-lysine doses from 0-800 mg in rats and 0.5-7.5 g in humans were analyzed for their effect on gastric emptying and gastrointestinal secretion. Human GI function was assessed non-invasively using magnetic resonance imaging (MRI), rat data were acquired using standard lethal measurement methods. L-lysine dose-dependently delayed gastric emptying and stimulated gastrointestinal secretion in rats as reflected by residual phenol red content and increased gastric wet weight. **Key Results:** The dose-dependent delay in gastric emptying observed in rats was confirmed in humans with an increase in half-time of gastric emptying of 4 min/g L-lysine, $p < 0.01$. Moreover, a dose-dependent increase in intestinal fluid accumulation was observed (0.4 ml/min/g L-lysine, $p < 0.0001$). No effect on alkaline tide, glucose concentration, hematocrit or visceral sensations was detected. **Conclusions:** This translational study demonstrates comparable dose-dependent effects of intragastric L-lysine on GI function in humans and rats and suggests a broader role for individual amino acids in the control of gastrointestinal motility and secretion *in vivo*.

Keywords

L-lysine, gastric emptying, gastrointestinal function, Magnetic Resonance Imaging

Key Messages

Why this paper is important

The essential amino acid L-lysine was demonstrated to have a selective impact on food intake, gastric emptying and intestinal transit in rats thus indicating a potential novel direct sensory input to assess dietary protein content and quality. This work represents an important translational extension of reported findings using magnetic resonance imaging (MRI) in human subjects and standard lethal measurements in rats. We show that intragastric L-lysine exhibits a comparable dose-dependent effect on GI motility and secretion in humans and rats. We strongly believe that these translational findings further support the broad role of specific amino acids in the control of GI function in vivo.

The aim of the research work

The aim of this study was to assess the translational aspects of recent findings in rats demonstrating a selective impact of L-Lysine on gastrointestinal function and to investigate the dose-dependent effect of L-lysine on human and rat GI function.

Basic methodology of study

L-lysine doses from 0-800 mg in rats and 0.5-7.5 g in humans were analyzed for their effect on gastric emptying and gastrointestinal secretion. Human GI function was assessed non-invasively using magnetic resonance imaging (MRI), rat data were acquired using standard lethal measurement methods.

Summarizing key results

A dose-dependent delay in gastric emptying was observed in humans with an increase in half-time of gastric emptying of 4 min/g L-lysine, $p < 0.01$. A dose-dependent increase in intestinal fluid accumulation was observed (0.4 ml/min/g L-lysine, $p < 0.0001$).

Introduction

The worldwide rapid increase in nutrition and in particular obesity related diseases stimulated efforts to understand how a meal impacts on various physiological processes including gastrointestinal (GI) function. Upon food intake, GI function adapts quickly to the ingested meal presumably to control efficient digestion and absorption of the vital nutrients. This dynamic plasticity is regulated by mechanisms responding to properties specific to the ingested meal such as their caloric content, volume and consistency. This multiple signaling input is integrated to some extent by the autonomous nervous and endocrine systems to control proper GI function (1).

The macronutrient composition of a meal is commonly not considered to have a major impact on GI function (2, 3). This is in contrast to newly emerging concepts demonstrating macronutrient specific control of different vagal and endocrine signals. For instance, specific individual amino acids induce vagal afferent firing or stimulate the release of different GI hormones *ex vivo* (4-8). The *in vivo* relevance of these findings in the context of GI function control has typically been extrapolated from studies showing that high protein diets delayed gastric emptying to a larger extent than isocaloric control diets in both humans and rats (9, 10). Controversially, others could not confirm these observations (11). As high protein diets are composed of different individual amino acids and thereby considerably vary between laboratories, this might obscure amino acid specific effects. So far, no study systematically tested the impact of all the 20 individual amino acids on GI function in humans or rats. Therefore the selection of candidate amino acids was based on a recent study in rats revealing that L-arginine, L-lysine and L-glutamic acid induced the most potent anorectic effect compared to isomolar doses of all other proteogenic amino acids (12). Within this study L-lysine was shown to delay solid gastric emptying, induce gastric secretion and increase intestinal motility thereby revealing an amino acid specific impact on GI function *in vivo*.

Several aspects remained elusive. Neither the dose-dependent effect nor the temporal dynamics of L-lysine were resolved, but both are relevant for the characterization of the pharmacokinetic effect of L-lysine. Most importantly, it remained unclear if humans show a similar gastrointestinal response to a gastric load of L-lysine. Humans and rodents significantly differ in GI anatomy and physiology even though from an ingestive perspective both are omnivores. For instance, rats eat 10-20 % of their body weight equivalent as food every day, whereas healthy humans at best 2 %. Moreover, rats do not have a gallbladder and lack the vomiting reflex.

The aim of this study was to identify the dose-dependent effect of L-lysine on human and rat GI function after intragastric infusion or gavage, respectively. Animal experiments were restricted to the 30 min time point due to lethal methods. GI function was analyzed by measurements of wet weight and residual phenol red content. In humans, magnetic resonance imaging (MRI) and venous blood sampling was applied to repeatedly assess gastric meal, secretion and intestinal fluid content volume as well as blood parameters every 15 min for over 90 min. Human experiments were performed in healthy volunteers within a randomized, double-blind, three-armed, cross-over study design. The study revealed a conserved impact of L-lysine on GI function in humans and rats.

Materials and Methods

Ethical approval

All procedures for rat handling and experimental interventions were according to the Swiss Animal Welfare laws and approved by the Kantonales Veterinäramt Zürich. The human study was performed between April and September 2012 in a single center, randomized, double-blind, 3-armed, unbalanced, cross-over study design and in accordance with the Declaration of Helsinki, Good Clinical Practice. It was registered at ClinicalTrial.gov with identifier NCT01212614. The local Ethics Committee approved the study and all volunteers gave written informed consent.

Animal care

30 male Wistar rats (Janvier, Saint Berthevin Cedex, France) were grouped housed (room temperature $21 \pm 1^\circ \text{C}$, artificial 12/12 h light dark cycle) with water and food –(3433 Kliba Nafag, Kaiseraugst, Switzerland) available ad libitum unless noted otherwise.

Animal dose preparation

0, 25, 50, 100, 200 and 400 mg/ml L-lysine (Sigma-Aldrich, Buchs, Switzerland) was solubilized in tap-water and 80, 75, 70, 60 and 40 mg/ml NaCl (Sigma) proportionally added to correct for molarity differences. pH was adjusted to 7.2 using HCl (Merck, Zug, Switzerland). The applied volume was 2 ml for each of these solutions supplemented with 1.5 mg phenol red (Sigma).

Animal experimental design

Animals were food deprived for 4 h prior oral L-lysine administration to ensure similar residual stomach content. Rats received intragastric application of 2 ml of the respective L-lysine dose and were returned to their home cage, where they did not have access to food or

water. 30 min post application, animals were euthanized using pentobarbital (IP, 100 mg/kg, Kantonsapotheke Zürich, Switzerland) in combination with isoflurane (5 %) for quicker induction. The GI tract was excised and segmented into stomach, small intestine, cecum and colon based on anatomical landmarks using clamps to limit liquid loss. The wet weight of each segment was immediately measured. Phenol red was extracted from each segment and quantified by optical spectroscopy at 560 nm wavelength as described previously (12).

Animal Statistics

Rats were randomly allocated into the six treatment groups. Application order was randomized and statistical analysis performed as described for the human data set below.

Human Subjects

Six healthy subjects aged between 18-50 years (four male, two female, BMI $22 \pm 1 \text{ kg/m}^2$) were recruited via a public advertisement. A T-test based power analysis was performed based on data from a previous study in 14 healthy subjects that measured the production rate of gastric secretion after intake of a secretion stimulating test meal (13). For six subjects, an effect size of 1.5 and selecting a significance level of 0.05 a power of 0.83 was calculated. Prior study inclusion, all subjects underwent a clinical assessment and were tested negative for *helicobacter pylori*. The following exclusion criteria were applied: BMI (kg/m^2) <19 or >24, history of systemic and psychiatric disease especially GI disease or surgery (excludes appendectomy, hernia repair and anorectal disorders), lysineuria, galactosaemia, medication intake of any kind, drug or alcohol abuse, presence of metallic implants, devices or metallic foreign bodies, claustrophobia, pregnancy and lactation, known allergy or intolerance against locus bean gum, L-lysine, Gadolinium (DOTAREM®), Methylparaben (E218), Fructose, Gluten, Galactose and also allergic reaction after prior injection of Lidocain (e.g. at the dentist).

Human Test Meal

L-lysine was administered as L-Lysine monohydrate (LMH, Fagron GmbH & Co.KG, Barsbüttel, Germany) at doses of 0.5, 1.2, 3.0 and 7.5 g in four different 300 ml test meals. The maximum L-lysine dose of 7.5 g was selected based on literature data. Doses up to 9 g are considered a physiological daily dose (14), however, doses > 8g have been reported to induce diarrhea and cramps. Lower doses were derived using a reduction factor of 2.5. The ingredients and properties of the four LMH test meals had negligible caloric content (<25 kcal) and are listed in **Table 1**. NaCl was proportionally added to each test meal to correct for molarity differences. All meals were prepared directly before each study session, heated to 37 °C and infused within 1-2 min while volunteers were lying in the MRI scanner.

Human measurement protocol

The human dose-response study was performed using a randomized, double-blind, 3-armed, unbalanced, cross-over study design. Three of the four L-lysine test meals were randomly assigned to the six subjects. This unbalanced study design was selected to receive maximum output while minimizing exposure. All six subjects underwent three study sessions of 2.5 hours including subject preparation and repeated MRI and venous blood measurements. Sessions were performed on three different days separated by 5 - 21 days. Each session followed the same procedure depicted in **Figure 1**. Subjects arrived at the MR center in the morning or at noon after fasting overnight (or for at least 8 h). A nasogastric tube (Freka® Tube CH/FR 12) was placed 50-60 cm from the nares after previous local anaesthesia of the nasal cavity with Lidocain-HCl 2 % (prepared in house) and Vibrocil® (Novartis, Basel, Switzerland) nose gel. A venous vascular cannula (Vasofix®Braunüle®) was placed on the right or left cubital vein for subsequent blood sampling and continuous 0.9 % NaCl infusion. MR image data were acquired with subjects positioned in right decubitus before and directly after test meal infusion and then every 15 min until 90 min. Visceral sensations scores were

recorded after each MRI scan and in addition at 105 min after meal infusion. Blood samples were collected before (baseline) and at 10, 25, 40, 55, 70, 85 and 100 after meal infusion. The first blood sample in fasted state and last blood samples at 100 min were taken in sitting position, all other samples were obtained while the subject was lying on the MR patient table. The nasogastric tube was removed at ~60 min with subjects in sitting position.

Human MRI and visceral sensations scores

Subjects were positioned in right decubitus inside the clinical MRI scanner (1.5 T Achieva, Philips Healthcare, Best, NL). An abdominal phased-array surface coil (SENSE body coil, four elements) was used for image acquisition. MR imaging consisted of three imaging sequences to visualize gastric content volume, gastric secretion, gallbladder volume and intestinal fluid content. The MRI scan parameters for gastric content volume measurement were: balanced steady state free precession sequence, 26 - 34 transverse slices, 6 mm slice thickness, no slice gap, 360 mm field of view, 1.6/3.3 msec echo/repetition time, one breath hold, 16 – 24 sec scan duration (depending on slice number). For assessing gastric secretion, MRI scan parameters were: radial T1-weighted gradient echo sequence with profiles acquired in golden angle view order, 18 coronal slices, 6 mm slice thickness, 0.6 mm slice gap, 360 mm field of view, 1.8/12 msec echo/repetition time, free breathing, 2:24 min scan duration. To visualize gallbladder volume and intestinal fluid content, MRI scan parameters were: T2-weighted turbo spin echo sequence with fat suppressions, 26 coronal slices, 5 mm slice thickness, 1 mm slice gap, 375 mm field of view, 80/1200 msec echo/repetition time, two breath holds, 31 sec scan duration.

After MRI scans, subjects had to report their visceral sensations, i.e. hunger, fullness, nausea, bloating, abdominal cramps and urge to defecate using a self-assessed sensations scale ranging from 0 – 10 (0 – no sensations, 10 – extremely prominent sensations).

Human blood sampling and analysis

For blood gas measurements assessing the alkaline tide (pH, bicarbonate, pCO₂, chloride), glucose as well as hematocrit, venous blood was first collected with a serum vacutainer, then filled within 1-3 minutes in heparinized capillaries and analyzed using the ABL 700 blood gas analyzer (Radiometer GmbH, Thalwil). For measurements of albumin and L-lysine blood concentration, Li-heparin vacutainers and EDTA-vacutainers were used, respectively. Albumin was measured to assess a potential shift in fluid from intravasal to intragastrointestinal. Blood samples were directly centrifuged and ~ 1 ml of plasma was pipetted for storage at -20 °C for L-lysine and 8 °C for albumin. 50 µl of each L-lysine plasma sample was mixed with 50 µl of 10 % SSA/NVal solution and centrifuged at 15'000 rpm for 5 min. 60 µl borate/NaOH buffer and 20 µl reagent (2AMT) was added to 20 µl of the resulting supernatant and 1/100 µl of this mixture was injected on a HPLC column using the cell culture gradient, according to the manufacture's protocol. The plasma for the albumin measurement was processed with a Cobas 8000 modular analyzer (c701 module, Roche, Mannheim) subsequent a color test method (Bromocresol green) with endpoint measure.

Data analysis

MR image analysis was performed blindly by a radiologist in training (TR) and a research assistant with one year of training in analyzing abdominal MR image data (CB). Purpose built software tools implemented in MATLAB (The MathWorks, Natick, MA) and IDL (Exelis Visual Information Solutions) were applied for extraction of gastric content volume, gastric secretion, gallbladder volume and intestinal fluid content. Gastric content contours were segmented in each transverse image by a semi-automated algorithm. Respective 3D gastric content volumes were computed from 3D isosurfaces of the contours (13). Gallbladder contours and small intestinal areas excluding blood vessels and visceral tissues were manually contoured in each T2-weighted coronal image slice. To assess intestinal fluid content, a signal

threshold was selected to segment the bright luminal fluid content from air and surrounding soft tissue. Intestinal fluid volume was calculated by summing all segmented pixels. Gastric secretion volume was analyzed from reconstructed T1 maps according to a previously established analysis method (13).

MR parameters were smoothed using function *loess* (with smoothing parameter $\alpha = 1$) in program R (16) and plotted over time and LMH dose. Half-times of gastric emptying (t_{50}) were calculated from smoothed curves by solving the locally smoothed interpolation for 50% using the Newton method (17).

Dose response of t_{50} was analyzed by linear mixed model with t_{50} and LMH dose as fixed effects and subject as random effect. Dose response of gallbladder and intestinal fluid volumes was analyzed by linear mixed model with volume, time and LMH dose as fixed effects and subject as random effect.

The density functions of individual mean visceral sensations scores were plotted. Since the resulting distributions were dominated by zero values, visceral sensations scores were divided into two categories, i.e. 0 and ≥ 1 , to only test for a dose dependent increase in scores not zero. The dose dependent effect of L-lysine on the binomial visceral sensations scores was analyzed using generalized linear models with a binominal link function.

Deltas over baseline (DOB) values were calculated for L-lysine and albumin concentrations, all blood gas parameters and base excess. Baseline was defined as the values detected at fasted state. DOB values were smoothed using function *loess* and plotted over time and LMH dose. Dose response was analyzed by linear mixed models with DOB of blood gas parameters, time and LMH dose as fixed effects and subject as random effect. Estimated model parameters are presented as estimate \pm standard error. A significance level of 0.05 was chosen.

Results

Animal experiments

To identify a dose dependent impact of L-lysine on rat GI function, we administered different isomolar doses of L-lysine intragastrically and measured GI tract wet weight and phenol red transit 30 min post-application. The doses applied ranged from 0–800 mg L-lysine per animal covering the span of daily L-lysine intake in rats (12). Rat stomach and cecum wet weight dose-dependently increased by 0.4 ± 0.04 g / 0.1 g L-lysine ($p < 0.0001$) and 0.2 ± 0.01 g / 0.1 g L-lysine ($p = 0.03$) respectively (**Figure 2 A, B**). No other GI segments showed a dose dependent change in wet weight. As animals did not have access to water or food, we interpreted these wet weights changes to reflect alterations in secretion or absorption, respectively. To access GI motility more specifically, we tracked the transit of a dye (phenol red) along the GI segments 30 min post application. By measuring residual phenol red in the stomach, we observed delayed gastric emptying by increased gastric phenol red content of $3.0 \pm 0.8\%$ per 0.1 g L-lysine ($p < 0.001$) and decreased small intestinal phenol red content of also $3.0 \pm 0.4\%$ per 0.1 g L-lysine ($p < 0.0001$, **Figure 3 A, B**). In accordance, colon phenol red content also decreased by $0.3 \pm 0.08\%$ per 0.1 g L-lysine ($p < 0.001$, **Figure 3 C**). The gastric and intestinal phenol red content appeared to plateau at the two higher L-lysine doses. These data show that L-lysine dose-dependently delayed gastric emptying and stimulated gastric secretion in rats. Additionally, cecum wet weight increased dose dependently indicating alterations in intestinal, pancreatic or biliary secretion. This data is limited to the 30 min time point thereby potentially obscuring lower dose effects of L-lysine.

Human experiments

To assess the translational aspects of L-lysine impact on GI function, we conducted a MRI based study in healthy human subjects. Briefly, we administered different isomolar doses of

L-lysine nasogastrically and measured the impact on GI function and blood gas parameters every 15 min over a time course of 90 min. The administered doses were between 0.5 g and 7.5 g L-lysine per test meal, which is considered physiological relevant as the daily L-lysine intake varies between 2 to 9 g/day (14). In one subject, L-lysine measurements for one dose had to be dismissed due to non-compliance with study procedure (subject P4, 3 g L-lysine dose). After L-lysine application blood L-lysine concentration increased dose-dependently over time by $0.54 \pm 0.1 \mu\text{M}/\text{min}/\text{g}$ L-lysine ($p < 0.0001$, **Figure 4 A, B**) demonstrating proper application and absorption. No dose dependent differences were found for albumin or any of the blood parameters. At the level of the stomach L-lysine dose dependently delayed gastric emptying by an increase in t_{50} of $4.2 \pm 1.4 \text{ min}/\text{g}$ L-lysine ($p = 0.01$, **Figure 5 A, B**). The infused test meals with 0.5 g and 1.2 g L-lysine emptied similar to a drink of 300 ml of water (18). No changes in gallbladder volume or gastric secretion were detected based on MRI. In the small intestine, L-lysine dose- and time-dependently increased fluid volume by $0.37 \pm 0.1 \text{ ml}/\text{min}/\text{g}$ L-lysine ($p < 0.0001$, **Figure 6 A, B**). This effect and its dynamics were clearly detectable in the MR image data (**Figure 7**). During the 100 min postprandial period, no side effects or dose dependent changes in self-reported visceral sensations were found. After end of study protocol, defecation was altered after the intake of 7.5 g L-lysine, as 4 of 5 subjects receiving this highest dose retrospectively reported self-limiting diarrhea within one to six hours after L-lysine application. No such change in defecation was reported for any other dose. In summary, these findings confirm the dose-dependent delay in gastric emptying and demonstrate an increase in intestinal liquid content in humans.

Discussion

This study revealed several similar functional responses of the GI tract to L-lysine stimulus in humans and rats. In both species, gastric and small intestinal functions were effectively altered after intragastric infusion of L-lysine.

L-lysine delayed gastric emptying revealing a conserved amino acid specific effect in rats and humans. This delay seemed not to trigger a temporal delay of L-lysine absorption into the blood stream in humans for enabling post-gastric regulatory mechanisms. Gastric emptying is generally accepted to be primarily controlled by the volume and the caloric equivalent of the gastric load (1). The two low dose test meals (0.5 g and 1.2 g) emptied faster or similar to a 300 ml drink of water (18), while the highest 7.5 g L-Lysine dose delayed the volume emptying by ~30 min. The negligible caloric differences between the lowest and highest L-lysine doses in humans (< 23 kcal) and also rats (< 5 kcal) suggest a caloric independent mechanism for the observed alterations in gastric emptying. This amino acid specific mechanism for the control of GI function has been recently proposed for L-lysine, L-arginine and L-glutamic acid in rats (12) and is herewith confirmed for humans. Beyond that, the former rat study showed that intravenous L-arginine and L-lysine did not modulate GI function indicating a potential GI luminal mechanism of action (12).

This work's focus was limited to the short term effect of L-lysine on gastrointestinal motor function in rats and humans. Our previous work in rats didn't suggest a long term effect, since L-lysine reduced food intake only in the first hour after application, but did not alter energy intake in the subsequent hours (19). Based on this anorectic effect we concluded that L-lysine acts in the short-term rather than in the long term. No simultaneous measurements of enteroendocrine hormones were performed that would have allowed to gain further insights in regard to the mechanism or site of action. Indeed, L-lysine might have stimulated the release of specific GI hormones. For instance the release of the gut hormone GLP-1 from primary

murine intestinal cells was shown to be induced by L-glutamic acids (20). Similar L-phenylalanine and L-tryptophan were reported to stimulate cholecystokinin (CCK) secretion from primary cells (21). These findings demonstrated the remarkable amino acid specificity for the secretory function of endocrine cells *ex vivo*. However, the specific impact of L-lysine on GI hormone release and their relevance for the modulation of GI function *in vivo* is currently unknown. The underlying regulatory mechanism of the L-lysine effect might have also involved vagal signaling. Several *ex vivo* electrophysiological studies revealed amino acid specificity of vagal firing as different amino acids excite or inhibit vagal afferent activity dependent on the exposed vagal branch (4-8). Interestingly of all amino acids tested only intragastric L-glutamic acid induced vagal signaling *ex vivo* indicating the necessity for amino acid absorption (22, 23). Hepatic vagal afferents fire specifically to an L-lysine stimulus and they were shown to mediate the anorectic effect of L-lysine in rats (8, 12). Taken together, this study showed that L-lysine dose dependently delayed gastric emptying suggesting a caloric independent mechanism for the control of GI function also in humans.

Gastric secretion was dose dependently induced in animals but not in humans. Importantly, delayed gastric emptying and secretion had a different dose response curve in rats. Hence, the L-lysine doses used here in humans might have been insufficient to extensively stimulate gastric secretion. A formerly more invasive study by Sackler et al. applying repeated extraction of gastric content with subsequent titration reported increased gastric pepsin and HCl secretion after application of 5 g L-lysine combined with a solid test meal (24). Hence, the test meal composition and the sampling method might have synergized with L-lysine to induce increased gastric secretion. It is understood, that gastric secretion is mainly mediated by parietal cells. Here, we did not assess if L-lysine stimulated parietal secretion directly or induced histamine secretion by enterochromaffin cells; however, because histamine not only induces gastric secretion but also delays gastric emptying this might be an alternative mediatory mechanism of the observed L-lysine action (25, 26). The lack of L-lysine's effect

on gastric secretion in humans was reflected and supported by the absence of any differences in blood gas parameters and base excess. Gastric secretion was previously shown to correlate with base excess in blood, an effect termed alkaline tide (27). Taken together, L-lysine induced gastric secretion at high doses in rats, and a similar effect might be probable in humans. However, its analysis is hampered by the diarrhea inducing side effect of L-lysine doses ≥ 7 g. The reported side effects of self-limiting diarrhea for the highest Lys dose of 7.5 g in humans were accompanied by an increase in intestinal fluid volume. In both humans and rats, a dose dependent increase in intestinal fluid volume, respectively cecum wet weight, was observed. The origin of this fluid accumulation is currently unclear, but might arise either from increased bile and pancreatic secretions, reduced luminal absorption and/or active intestinal fluid influx. A simple osmotic effect seems unlikely, because we applied isomolar L-lysine solutions. Since no dose dependent effect on gallbladder volume was detected in humans, and because rats have no gallbladder the latter two possibilities are currently the more likely reasons for this phenomenon.

In healthy humans, the apparent impact of L-lysine on gastric and small intestinal function did not translate into changes in visceral sensations scores. Hunger and fullness were the only scores to exhibit density functions with modes different from zero. Therefore, a simplified ordinal, i.e. binomial, model analysis had to be applied to test for a dose dependent increase in scores ≥ 1 . This finding might not be surprising given the low numbers of study participants and the multitude of determinants of visceral sensitivity (28) . A larger number of volunteers would be needed to conclude on the effect of L-lysine on human satiation.

The here presented work focused on liquid emptying. This may be regarded as methodological simplification in so far as animals and humans normally ingest solid and semi-solid meals, respectively. However, it is important to acknowledge that after a so called “lag phase” solid meals empty at a similar rate as liquid meals of similar composition and energy content (29). Within this initial phase of gastric emptying, secretion and motor activity

process the ingested food into a liquid like dispersion that can pass through the pyloric opening. Hence, the focus on liquid emptying is also reasonable from a physiological perspective (30, 31). This is also supported by previous data in rats showing delayed solid gastric emptying at a L-lysine dose of 6.7 mmol/kg (19). Within the human setting, liquid gastric emptying has become of more interest also in the clinical setting and is currently evaluated as a method for the detection of gastroparesis (32-35). In conclusion, this translational study demonstrates comparable dose-dependent effects of intragastric L-lysine on GI function in humans and rats, in particular stomach and small intestinal function. This conserved chemospecificity supports a broader role for individual amino acids in the control of gastrointestinal transport and secretion *in vivo*.

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Disclosures

All authors declare no competing financial, personal, or professional interest that could be construed to have influenced this paper.

Author contribution

All authors contributed to study design, data interpretation and manuscript revision. C.B performed all human experiments with the help of T.R. and A.S. J.J. performed all rat experiments with the help of B.H. J.J and C. B. wrote the paper with the help of F.V., T.A.L and A.S. All aspects of the project were supervised by F.V, T.A.L and A.S. All authors contributed to manuscript editing and approved the final version of the manuscript. All authors qualifying for authorship are listed.

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Tables

Table 1

Ingredients and properties of the four L-lysine test meals for human application.

Ingredients/properties	units	values			
LMH	[g]	0.5	1.2	3.0	7.5
NaCl	[g]	1.24	1.12	0.8	0.0
Evian® (still water)	[ml]	300			
Locus beam gum (E410)	[g]	3			
MR contrast agent (Dotarem®)	[μl]	350 μl(\triangleq 175 μmol \triangleq 583.3 μM)			
Osmolarity	[mOsm/l]	152.52			
pH	pH	9.2	9.6	9.6	9.85
Caloric load	[kcal]	2.3	4.5	10.4	25.1

Figures

Figure 1

Schematic of the human study protocol. The schematic depicts the sequence of MRI measurements, venous blood sampling and visceral sensation ratings as performed in all three study sessions.

Figure 1

O = **venous blood samples**

L-lysine, albumin, pH, bicarbonate, pCO₂, chloride, glucose, haematocrit

+ = **visceral sensations scores**

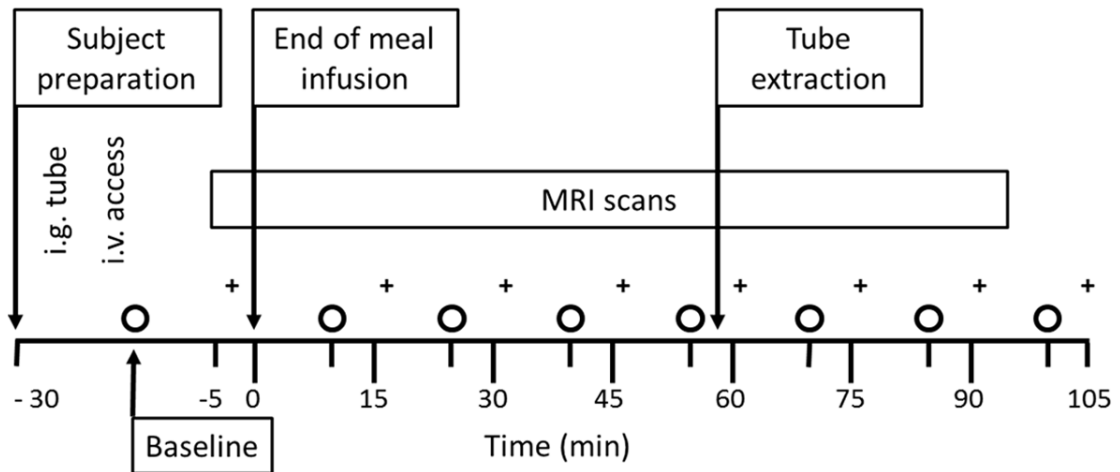


Figure 2

Dose dependent effect of intragastric L-lysine administration on GI wet weight in rats.

L-lysine dose-dependently increased stomach (A) and caecum wet weight (B) in rats.

Individual points represent wet weight measurements of individual animals treated with the respective L-lysine dose. The predicted linear dose-response curve is presented as solid line.

L-lysine doses (x-axis) are plotted in log₂.0 scale.

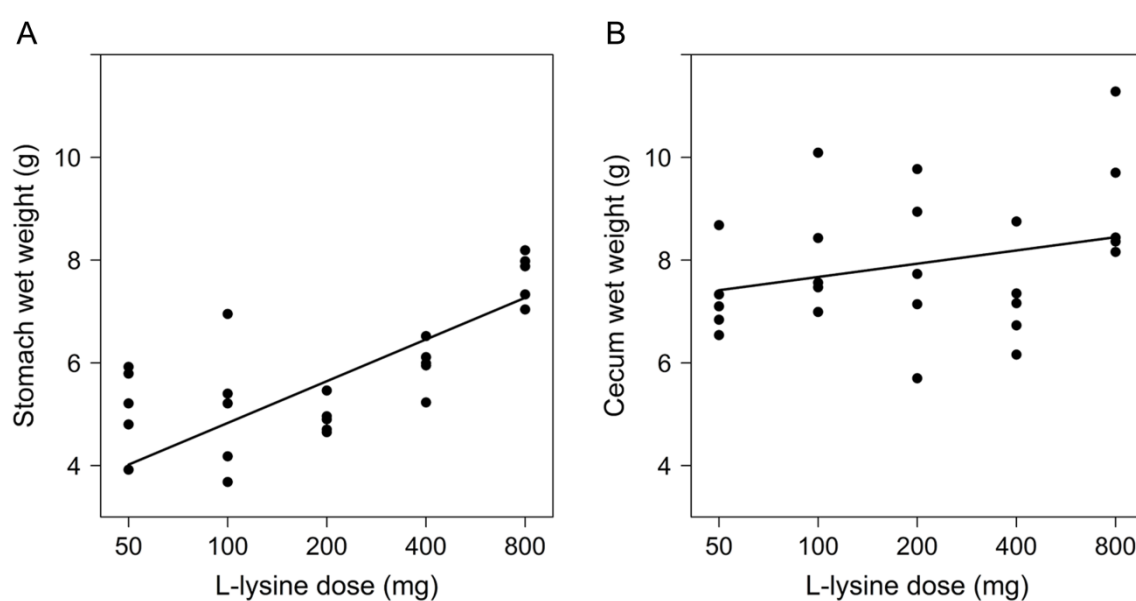


Figure 3

Dose dependent effect of intragastric L-lysine administration on phenol red content in rats. Phenol red content is expressed as percentage of the initially administered total phenol red amount. L-lysine dose-dependently increased gastric phenol red content (A) and decreased small intestinal (B) and colon (C) phenol red content in rats. Individual points represent residual phenol red content measurements of individual animals treated with the respective L-lysine dose. The predicted linear dose-response curve is presented as solid line. L-lysine doses (x-axis) are plotted in log₂ scale.

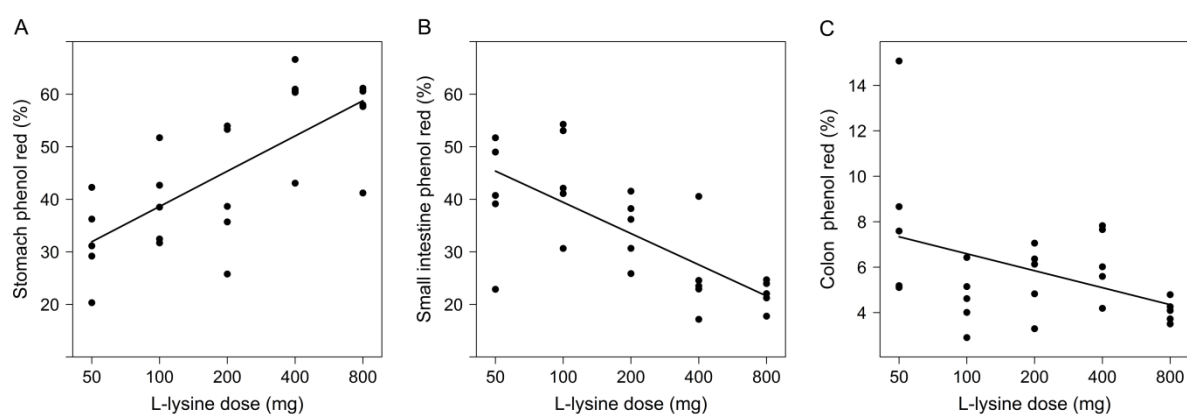


Figure 4

Dose dependent L-lysine concentrations in venous blood after intragastric application of L-lysine in humans. Data is expressed as delta over baseline (DOB) with baseline values measured before intragastric L-lysine infusion. (A) Smoothed DOB L-lysine concentration curves (lines) overlaid on the individual measurements (points) of all human subjects each treated with three (of the total four) different L-lysine doses. L-lysine concentration data of one dose in subject P4 was dismissed due to non-compliance of study protocol. (B) The average linearly predicted L-lysine concentration increase per dose.

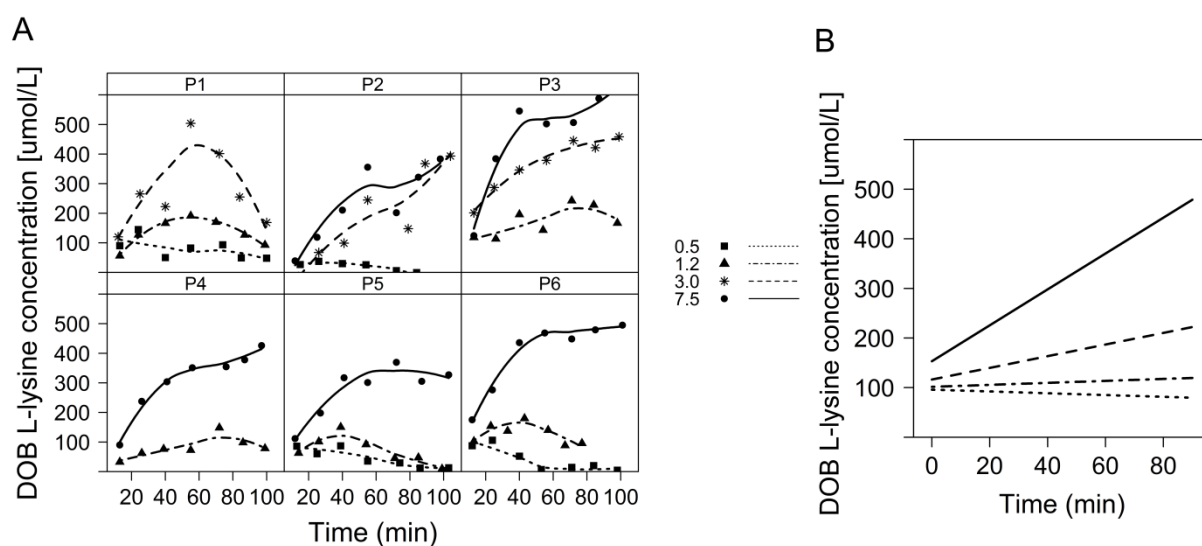


Figure 5

Dose dependent effect of intragastric L-lysine application on gastric emptying in humans. (A) Smoothed gastric emptying curves (lines) overlaid on the individual volume measurements (points) of all human subjects each treated with three (of the total four) different L-lysine doses. (B) L-lysine dose-dependently increased t50 (computed from smoothed gastric emptying curves) in humans. The predicted linear increase is indicated as solid line. Values of t50 (y-axis) are plotted in log10 scale, L-lysine doses (x-axis) are plotted in log2.5 scale.

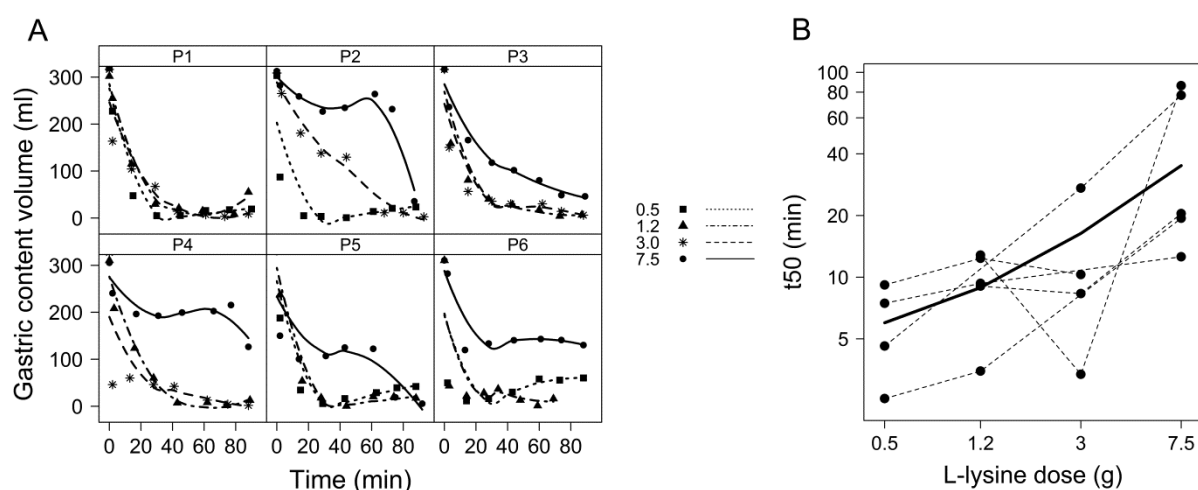


Figure 6

Dose dependent effect of intragastric L-lysine application on intestinal fluid volume in humans. (A) Smoothed intestinal fluid volume curves (lines) overlaid on the individual volume measurements (points) of all human subjects each treated with three (of the total four) different L-lysine doses. (B) The average linearly predicted intestinal fluid volume change per dose.

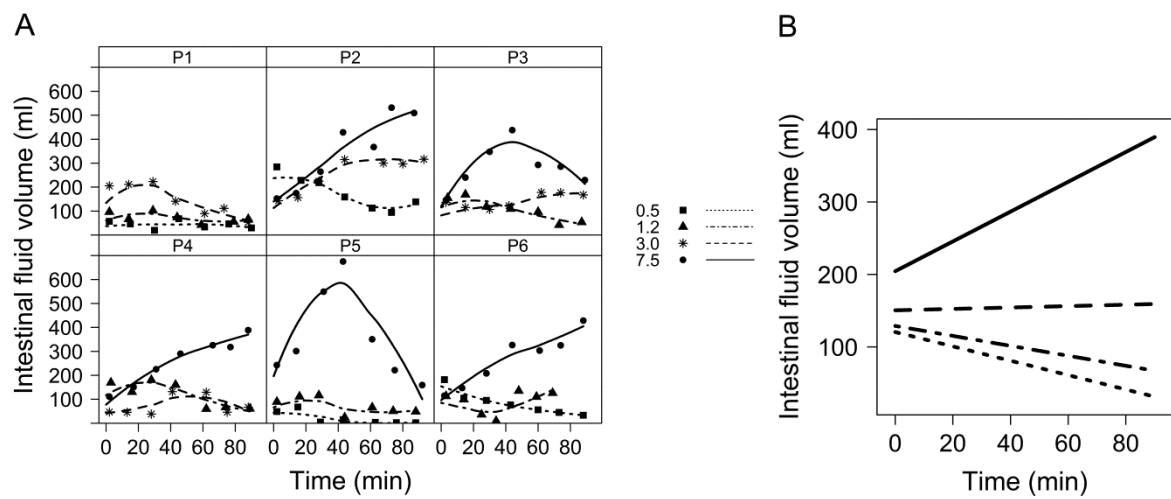
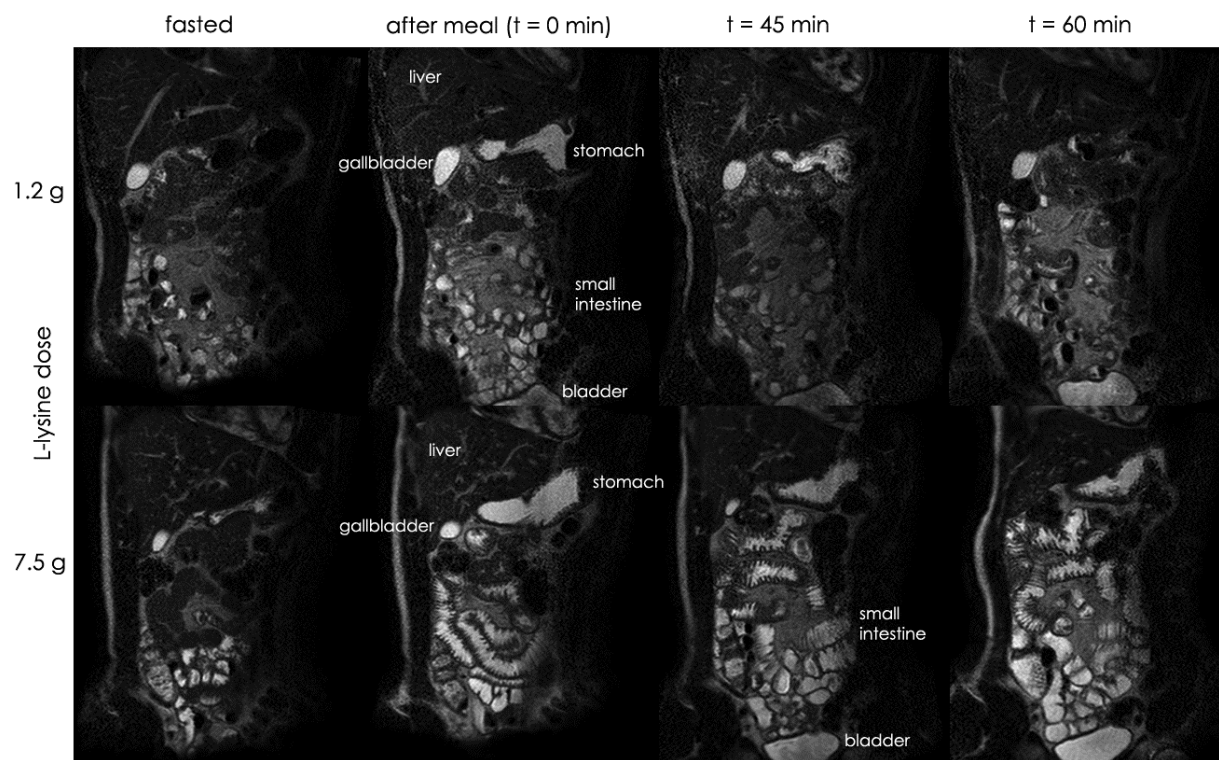


Figure 7

MR image data highlighting the dose dependent effect of intragastric L-lysine application on gastrointestinal fluid volume in humans. The difference in the accumulation of intestinal fluid volume and the delay in gastric emptying between L-lysine doses 1.2 g and 7.5 g is depicted over 60 min in representative MR image data of one subject (P6). Imaged GI organs and anatomical landmarks are indicated.

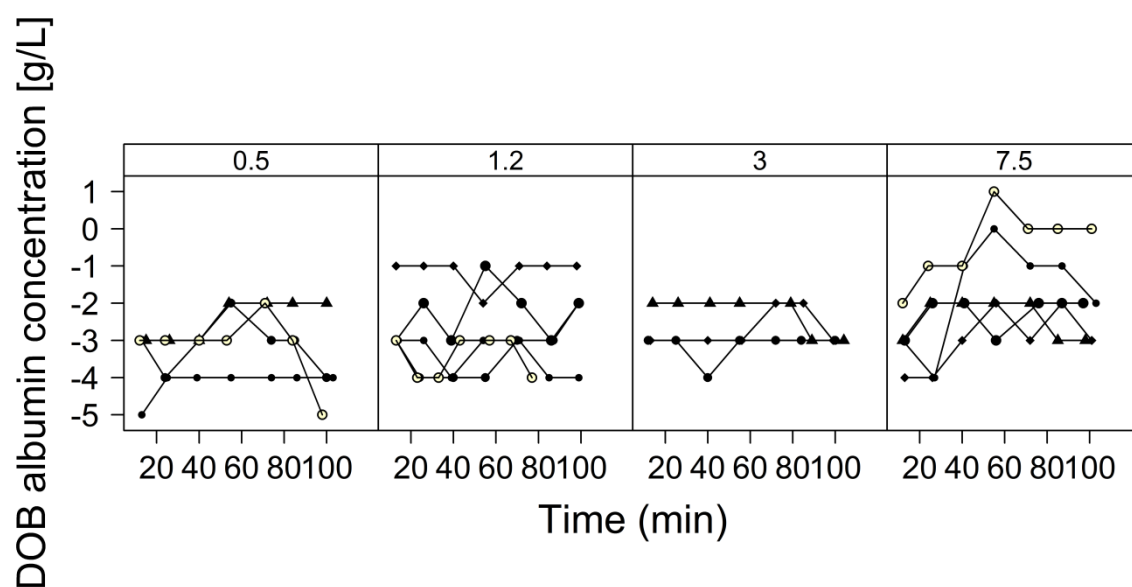


Supporting material

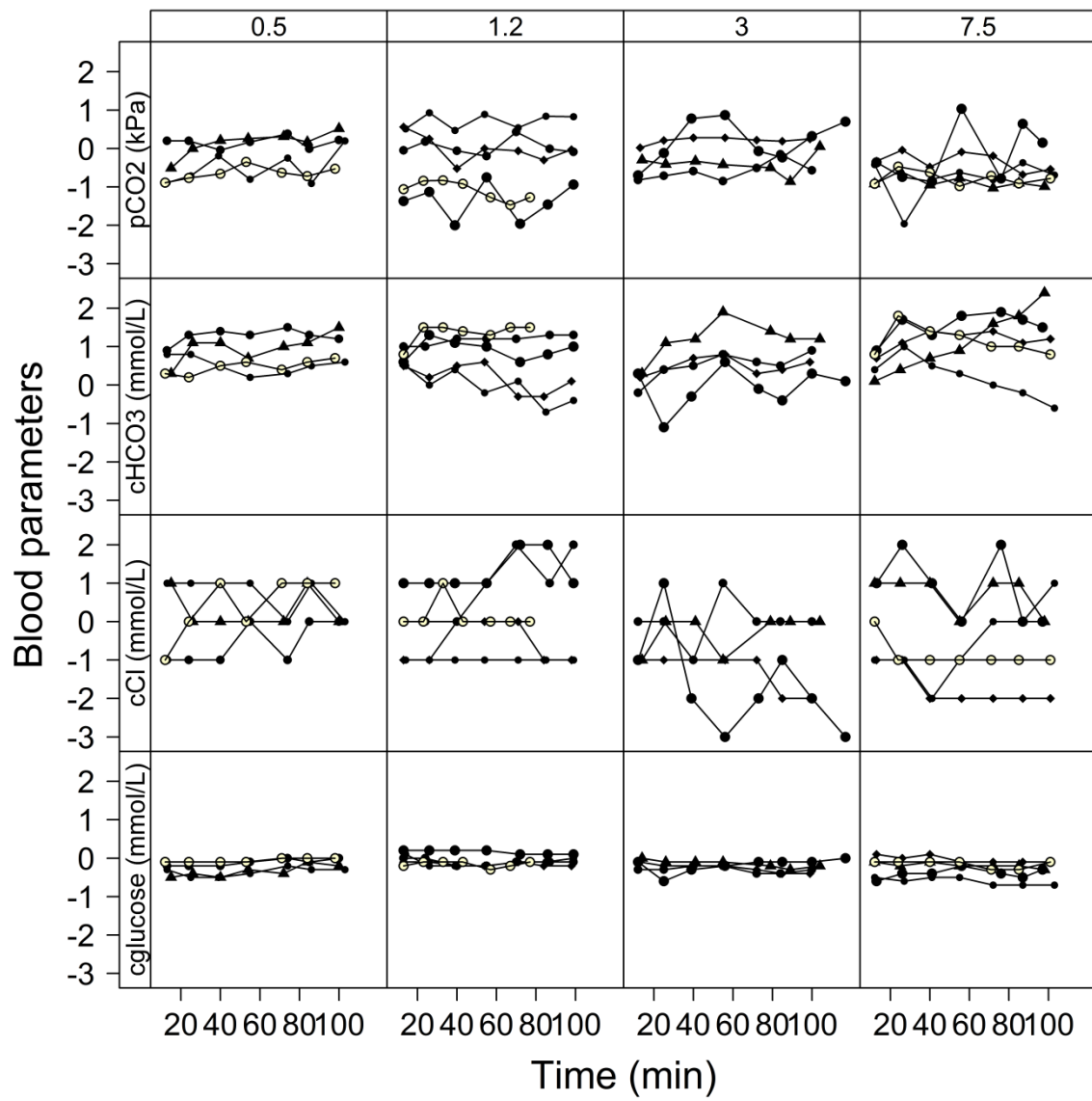
Figure S1

Albumin concentration and blood gas parameters from venous blood after intragastric L-lysine application in humans. Data are expressed as delta over baseline (DOB) with baseline values measured before intragastric L-lysine infusion. Data are grouped by dose into separate panels and individual volunteers are marked by different symbols. (A) Unsmoothed DOB albumin concentration curves (lines) overlaid on the individual measurements (points) per L-lysine dose. Dose dependent DOB albumin concentrations. (B) Unsmoothed DOB pCO_2 and bicarbonate, chloride and glucose concentration curves (lines) overlaid on the individual measurements (points) per L-lysine dose. (C) Unsmoothed DOB pH and haematocrit curves (lines) overlaid on the individual measurements (points) per L-lysine dose. No dose dependent effect of intragastric L-lysine application was observed in any of these blood parameters.

A



B



C

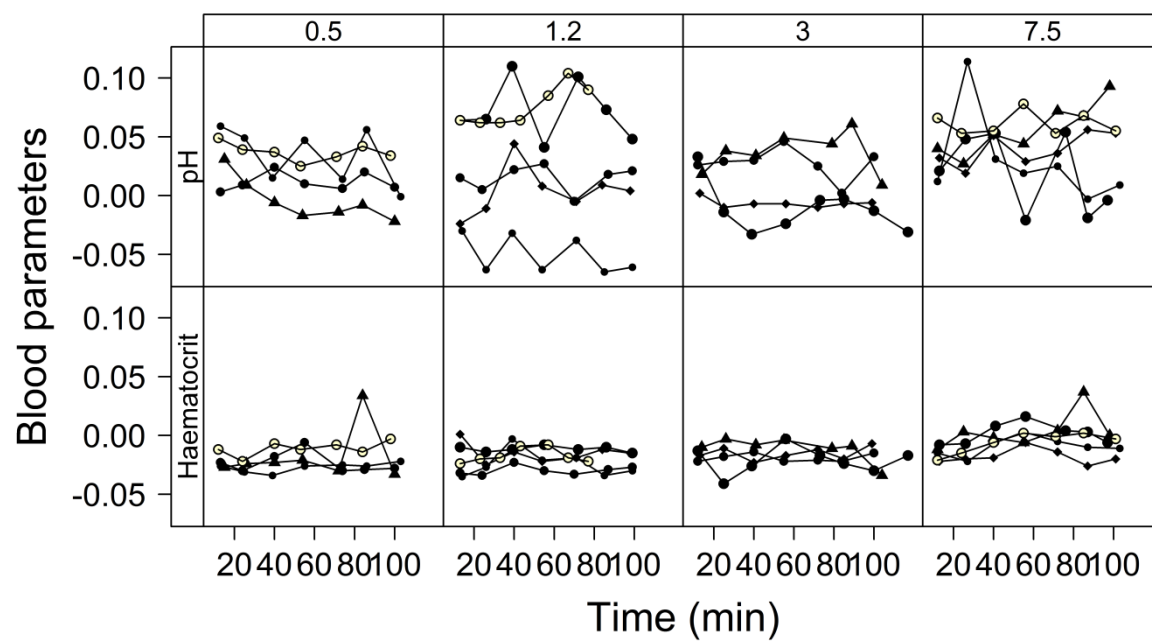
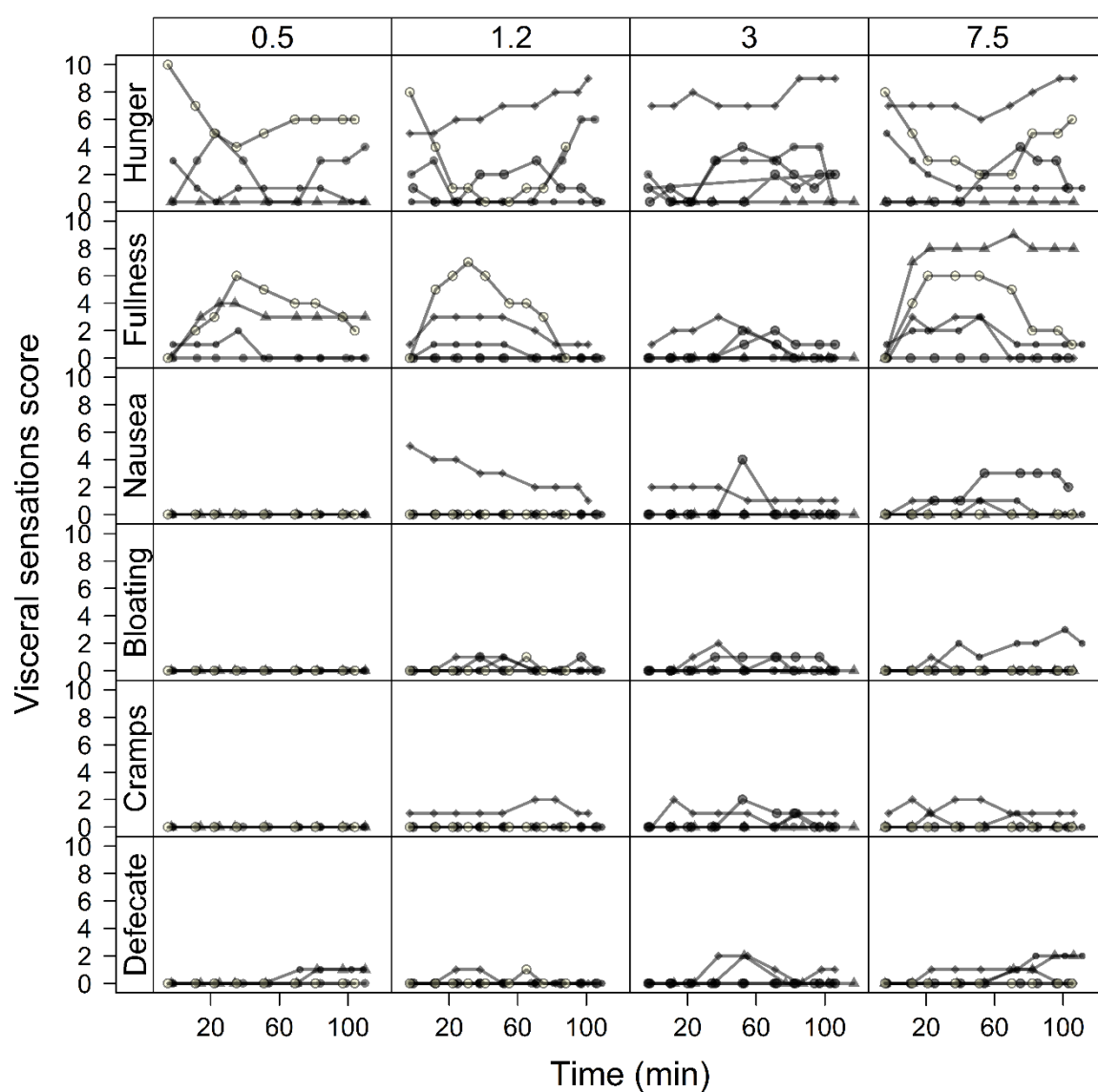


Figure S2

Visceral sensations scores after intragastric L-lysine application in humans. (A) Visceral sensation scores of all subjects plotted over time and dose. Only hunger and fullness scores showed a significant number of non-zero values. Because of the zero dominated and therefore skewed density functions visceral sensations scores were simplified to binomial data with categories 0 and ≥ 1 .

A



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